Amendments to the Specification

In the Title

Please amend the title as follows:

STEM CELL FACTOR COMPOSITIONS ~~CLAIMS~~

In the Specification:

Please update the priority information as follows:

At page 1, replace paragraph 1, beginning line 3 as follows:

At paragraph 1, page 1, beginning line 3:

--This is a divisional of U.S. Application Serial No. 09/005,893, filed January 12, 1998, which is a divisional application of 08/449,653, which was filed May 24, 1995, now U.S. Patent No. 6,248,319 issued June 19, 2001, which is a divisional application of U.S. application Serial No. 08/172,329 filed December 21, 1993, now U.S. patent No. 6,218,148 issued April 17, 2001, which is a continuation of U.S. application Serial No. 07/982,255 filed November 25, 1992, now U.S. patent No. 6,204,363 issued March 20, 2001, which is a continuation of U.S. application Serial No. 07/684,535 filed April 10, 1991, now abandoned, which ~~This~~ is a continuation-in-part application of ~~Serial No. 589,701.~~ U.S. application Serial No. 07/589,701 filed October 1, 1990, now abandoned, which is a continuation-in-part application of ~~Ser. No. 573,616~~ U.S. application Serial No. 07/573,616 filed August 24, 1990, now abandoned, which is a continuation-in-part application of ~~Ser. No. 537,198~~ U.S. application Serial No. 07/537,198 filed June 11, 1990, now abandoned, which is a continuation-in-part application of ~~Ser. No. 422,383~~ U.S. application Serial No. 07/422,383 filed October 16, 1989, now abandoned, each of which are hereby incorporated by reference.--

Page 9, line 10 please amend the paragraph as follows:

--FIG. 11 shows the amino acid sequence (SEQ ID NO.: 1) of mammalian SCF derived from protein sequencing.--

At page 9, lines 14-16 please amend the description of Figure 12 as follows:

--FIG. 12 shows

A. oligonucleotides for rat SCF cDNA (SEQ ID NOS.: 2-19)

B. oligonucleotides for human SCF DNA (SEQ ID NOS.: 20-30)

C. universal oligonucleotides. (SEQ ID NOS.: 31-38). --

At Page 9, line 26 please amend the figure legend for Figure 14B as follows:

--B. the nucleic acid sequence of rat (SEQ ID NOS.: 39 and 40) genomic DNA.--

At Page 9, line 29 please amend the figure legend to Figure 14C as follows:

--C. the nucleic acid sequence of rat SCF cDNA and amino acid sequence of rat SCF protein. (SEQ ID NOS.: 41 and 42)--.

At Page 9, line 34 please amend the figure legend for Figure 15B as follows:

--B. the nucleic acid sequence of human (SEQ ID NO.: 43 and 44) genomic DNA--.

At Page 10, line 1 please amend the figure legend for Figure 15C as follows:

--C. the composite nucleic acid sequence of (SEQ ID NOS.: 45 and 46) human SCF cDNA and amino acid sequence of SCF protein. --

At Page 10, line 4 please amend the figure legend for Figure 15D was follows:

--D. the nucleic acid sequence of genomic DNA and amino acid sequence of human SCF protein, including (SEQ ID NOS.: 47 and 48) flanking regions and introns. --

Page 10, line 8 please amend the figure legend for Figure 16A and B as follows:

--FIGS. 16A and B shows the aligned amino acid sequences of human, monkey, dog, mouse, and rat (SEQ ID NOS.: 49-57) SCF protein. --

Page 10, line 15 please amend the figure legend for figure 16D as follows:

--FIG. 16D shows the nucleotide sequence of the 221 base pair EcoRI-BamHI fragment constructed from (SEQ ID NOS.: 58 and 59) oligonucleotides that were used in creating the plasmid for human [Met^{-1}] SCF^{1-165}. Uppercase letters below the nucleotide sequence represent the amino acid sequence. Lowercase letters above the nucleotide sequence show nucleotides in the original $hSCF^{1-183}$ sequence that were altered to generate codons most frequently used by E. coli.--

At page 11, lines 24-25

--Figure 24 shows the effect of recombinant rat SCF on curing the macrocytic anemia of Steel mice, as assessed by hematocrit analysis (24A) or mean red blood cell volume (24B).--

At page 12, lines 4-6

--Figure 29 shows the effect of recombinant human sequence SCF treatment of normal primates in increasing WBC count.

29A. expressed as white blood cells in [K/cmm]

29B. expressed as peripheral blood cells in [K/cmm].--

At page 12, lines 8-10

--Figure 30 shows the effect of recombinant human sequence SCF treatment of normal primates in increasing hematocrits (30B) and platelet numbers (30A).--

At page 13, lines 26-27

--Figures 42A-42D shows human SCF cDNA sequence (SEQ ID NOs:60 AND 61)obtained from the HT1080 fibrosarcoma cell line.--

At page 13, lines 33-34

--Figures 44A-44C shows human SCF cDNA sequence (SEQ ID NOs:62 AND 63) obtained from the 5637 bladder carcinoma cell line.--

At page 15, lines 10-11

--Figure 56 shows 5-FU effect on ACH+ cells in marrow (56A) and spleen (56B).

At page 24, paragraph 2, beginning line 21

--Isoforms of SCF are isolated using standard techniques such as the techniques set forth in commonly owned U.S. Serial No. 421,444, entitled Erythropoietin Isoforms, filed October 13, 1989, now abandoned, hereby incorporated by reference.--

Page 49, please replace the paragraph beginning at line 13 with the following paragraph which provides a sequence identifier number for the sequence following the paragraph:

--SCF (purified as in Example 1; 400 pmol) in 50 mM sodium phosphate buffer (pH 7.6 containing dithiothreitol and EDTA) was incubated with 1.5 units of calf liver pyroglutamic acid aminopeptidase (pE-AP) for 16 h at 37° C. After reaction the mixture was directly loaded onto the protein sequencer. A major sequence could be identified through 46 cycles. The initial yield was about 40% and repetitive yield was 94.2%. The N-terminal sequence of SCF including the N-terminal pyroglutamic acid is SEQ ID NO.: 64:--.

At Page 51, please replace line 7 with the following line 7, which provides the sequence identifier for the sequence in that line:

--CB-6[1] 22.1 a. I-T-L-N-Y-V-A-G-(M) (SEQ ID NO.: 65)--

At Page 51, please replace lines 8-9 with the following lines 8-9, which provides the sequence identifier for the sequence at the end of line 9:

--b. V-A-S-D T-S-D-C-V-L-S- - -L-G-P-E-K-D-S-R-V-S-V-(_)-K---- (SEQ ID NO.: 66)--.

At Page 51, please replace line 11 with the following line 11, which provides the sequence identifier for the sequence at the end of line 11:

--CB-8 28.0 D-V-L-P-S-H-C-W-L-R-D-(M) (SEQ ID NO.: 67)--.

At Page 51, please replace lines 14-15 with the following lines 14-15, which provide the sequence identifier for the sequence at the end of line 15:

--CB-15[2] 43.0 E-E-N-A-P-K-N-V-K-E-S-L-K-K-P-T-R-(N)-F--T-P-E-E-F-F-S-I-F-D[3]-R-S-I-D-A------(SEQ ID NO.: 68)--

At Page 52, please replace line 17 with the following line 17, which provide the sequence identifier for the sequence at the end of line 17:

--T-1 7.1 E-S-L-K-K-P-E-T-R (SEQ ID NO.: 69)--.

At Page 52, please replace line 18 with the following line 18, which provides the sequence identifier for the sequence at the end of line 18:

--T-2[1] 28.1 V-S-V-(_)-K (SEQ ID NO.: 70)--.

At Page 52, please replace line 19 with the following line 19, which provides the sequence identifier for the sequence at the end of line 19:

--T-3 32.4 I-V-D-D-L-V-A-A-M-E-E-N-A-P-K (SEQ ID NO.: 71)--.

At Page 52, please replace line 20 with the following line 20, which provides the sequence identifier for the sequence at the end of line 20:

--T-4[2] 40.0 N-F-T-P-E-E-F-F-S-I-F-(_)-R (SEQ ID NO.:72)--.

At Page 52, please replace lines 21-22 with the following line 21-22, which provide the sequence identifier for the sequence at the end of line 22:

--T-5[3] 46.4 a. L-V-A-N-L-P-N-D-Y-M-I-T-L-N-Y-V-A-G-M-D-V-L-P-S-H-C-W-L-R (SEQ ID NO.: 73)--.

At Page 52, please replace lines 23-24 with the following line 23-24, which provide the sequence identifier for the sequence at the end of line 24:

-- b. S-I-D-A-F-K-D-F-M-V-A-S-D-T-S-D-C-V-L-S-(_)-(_)-L-G---- (SEQ ID NO.: 74)--.

At Page 52, please replace lines 25-26 with the following line 25-26, which provide the sequence identifier for the sequence at the end of line 26:

--T-7[4] 72.8 E-S-L-K-K-P-E-T-R-(N)-F-T-P-E-E-F-F-S-I-F-(_)-R (SEQ ID NO.: 75)--.

At Page 52, please replace lines 27-28 with the following line 27-28, which provide the sequence identifier for the sequence at the end of line 28:

--T-8 73.6 E-S-L-K-K-P-E-T-R-N-F-T-P-E-E-F-F-S-I-F-D-R (SEQ ID NO.: 76)--.

At Page 53, please replace line 16 with the following line 16, which provides the sequence identifier for the sequence at the end of line 16:

--S-1 5.1 N-A-P-K-N-V-K-E (SEQ ID NO.: 77)--.

At Page 53, please replace line 17 with the following line 17, which provides the sequence identifier for the sequence at the end of line 17:

--S-2[1] 27.7 S-R-V-S-V-(_)-K-P-F-M-L-P-P-V-A-(A) (SEQ ID NO.: 78)--.

At Page 53, please replace lines 20-21 with the following lines 20-21, which provide the sequence identifier for the sequence at the end of line 21:

--S-5[3] 71.0 S-L-K-K-P-E-T-R-N-F-T-P-E-E-F-F-S-I-F-(N)-R-SI-D-A-F-K-D-F-M-V-A-S-D (SEQ ID NO.: 79)--.

At Page 53, please replace lines 23-24 with the following lines 23-24, which provide the sequence identifier for the sequence at the end of line 24:

--S-6[3] 72.6 S-L-K-K-P-E-T-R-N-F-T-P-E-E-F-F-S-I-F-(N)-R-S-I-D-A-F-K-D-F-M-V-A-S-D-T-S-D (SEQ ID NO.: 80)--.

Page 54, please replace the paragraph beginning at line 3 and ending at line 14 with the following paragraph which has been amended to insert sequence identifier information for the sequence following the paragraph:

--SCF (2 μg) in 10 mM ammonium bicarbonate was dried to completeness by vacuum centrifugation and then redissolved in 100 μl of glacial acetic acid. A 10-20 fold molar excess of BNPS-skatole was added to the solution and the mixture was incubated at 50°C for 60 min. The reaction mixture was then dried by vacuum centrifugation. The dried residue was extracted with 100 μl of water and again with 50 μl of water. The combined extracts were then subjected to sequence analysis as described above. The following sequence (SEQ ID NO:81) was detected: --

Page 55, please rewrite lines 28-32 as follows to insert the sequence identifier information for the sequences on lines 29 and 31:

-- For example, cleavage ceases if the sequence Pro-Val is encountered. Peptide S-2 has the sequence S-R-V-S-V-(T)-K-P-F-M-L-P-P-V-A-(A) (SEQ ID NO.: 82) and was deduced to be the C-terminal peptide of SCF (see Section J in this Example). The C-terminal sequence of ---P-V-A-(A) (SEQ ID NO.: 83) restricts the protease cleavage to alanine only. The--

At Page 64, please replace the paragraph the begins at line 18 and ends at line 28 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--The sequence of the rat SCF coding region downstream of codon 162 was obtained by direct sequencing of the products of PCRs in which rat SCF (+)-strand primers were combined with (-)- strand primers designed from the human SCF 3'-untranslated region sequence. Rat SCF primers 224-24 (SEQ ID NO.: 10) (FIG. 12A) or 227-31 (5'-CCTGAGAAAGATTCCAGAGTC-3') (SEQ ID NO.: 84) were used in combination with either of the two human SCF primers 283-19 (5'-CTGCAGTTTGTATCTGAAG-3') (SEQ ID NO.: 85) or 283-20 (5'-CATATAAAGTCATGGGTAG-3') (SEQ ID NO.: 86). The rat SCF cDNA sequence is shown in FIG. 14C. --

At Page 75, please replace the paragraph the begins at line 20 and ends at line 32 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--To isolate a clone of exon 1 of the human SCF gene, a second genomic library was screened. The library, purchased from Clontech (Palo Alto, Calif.; catalog #HL 1067 J), was constructed in bacteriophage lambda vector EMBL3 SP6/T7 and contained 2.5 x 10^6 independent clones with an average insert size of 15 kb. Approximately 10^6 clones were plated and screened as described above using oligonucleotide probe 249-31 (5'-ACTTGTGTCTTCTTCATAAGGAAAGGC-3) (SEQ ID NO.: 87). A SacI restriction

fragment of the lambda clone was cloned into plasmid vector pGEM4 for subsequent sequence analysis. The sequence of the human SCF gene including exons 1, 7, 8 and 9 is shown in FIG. 15D.--

At Page 80, please replace the paragraph the begins at line 16 and ends at line 37 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--PCR amplification with primer 227-29 and one of the primers 227-30, 237-19 or 237-20 in each case except chicken yielded a fragment of the expected size which was sequenced either directly or after cloning into V19.8 or a PGEM vector. Additional sequences near the 5' end of the SCF cDNAs were obtained from PCR amplifications utilizing an SCF-specific primer in combination with either 254-9 or one of the non-specific primers 228-29 and 221-11. Additional sequences at the 3' end of the SCF coding regions were obtained after PCR amplification of 228-28 primed cDNA with combinations of SCF coding region (+)-strand primers with (-)-primers based on the human SCF 3' untranslated region as described in Example 3A. The primers 283-19 (SEQ ID NO.: 85) and 283-20 (SEQ ID NO.: 87) (Example 3A) and primer 287-9 (5'-TGTACGAAAGTAACAGTGTTG-3') (SEQ ID NO.: 88) were used. In the case of chicken, amplification was accomplished with primers to 227-29 (SEQ ID NO.: 14) or 247-1 (5'-ACTGCTCCTATTTAATCCTCTC-3') (SEQ ID NO.: 89) in combination with 247-2 (5'-CACTGACTCTGGAATCTTTCTCA-3') (SEQ ID NO.: 90) or 287-9. The aligned amino acid sequences of human (FIG. 42), monkey, dog, mouse, rat, cat, cow and chicken. SCF mature proteins are shown in FIG. 16.--

At page 85, paragraph 1, beginning line 1:

--Vector pDSVE is described in commonly owned U.S. Ser. Nos. 025,344, now U.S. Patent No. 5,175,255 issued Dec. 12, 1992, and 152,045, now abandoned, hereby incorporated by reference. The vector portion of V19.8 and pDSVE.1 ColE1 origin of replication and ampicillin resistance gene and the SV40 origin of replication. This overlap

may contribute to homologous recombination during the transformation process, thereby facilitating co-transformation.--

At Page 87, please replace the one-line paragraph on line 7 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--5'-TCTTCTTCATGGCGGCGGCAAGCTT-3' (SEQ ID NO.: 93)--.

At Page 89-90, please replace the paragraph bridging page 89 and 90 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--To determine the precise C-terminal processing site(s), the purified material was subjected to AspN peptidase digestion (20-50 µg SCF in 100-200 µl 0.1 M sodium phosphate, pH 7.2, for 18 h at 37°C with AspN:SCF ratio of 1:200 by weight) followed by HPLC to isolate resulting peptides. The elution profile shown in FIG. 16C was obtained. Collected peptide fractions were sequenced to identify the C-terminal peptide. A peptide eluting at 36.8 min represents the C-terminal peptide. The sequence Asp-Ser-Arg-Val-Ser-Val-(X)-Lys-Pro-Phe-Phe-Met-Leu-Pro-Pro-Val-Ala-(Ala) (SEQ ID NO.: 94) was assigned, where (X) denotes an unassigned residue, and (Ala) denotes tentative assignment due to low recovery. The indicated amino acids corresponds to position 148-165 of the sequence shown in FIG. 42. --

At Page 90, please replace the paragraph beginning at line 13 and ending at line 23 with the following paragraph which has been amended to add sequence identifier information to the paragraph:

--After treatment of the C-terminal peptide with neuraminidase and O-glycanase to remove carbohydrate, fast atom bombardment - mass spectroscopy (FAB-MS) analysis indicated a molecular weight of 1815.19 for the protonated monoisotopic ion (NH^+), consistent with the sequence Asp-Ser-Arg-Val-Ser-Val-Thr-Lys-Pro-Phe-Phe-Met-Leu-Pro-Pro-Val-Ala-Ala (SEQ ID NO.: 95) (calculated molecular weight of MH^+ =1815.98). A less

abundant ion species of mass 1744.37, corresponding to the above-mentioned peptide truncated by one Ala at the C-terminus (calculated MH$^+$ -1744.17), was also detected.--

At Page 91, please replace the text beginning at line 20 and ending at line 21 with the following text which has been amended to add sequence identifier information for the sequences following the paragraph:

--This example relates to expression in E. coli of SCF polypeptides by means of a DNA sequence encoding [Met^{-1}] rat SCF$^{1-193}$ (FIG. 14C). Although any suitable vector may be employed for protein expression using this DNA, the plasmid chosen was pCFM1156 (FIG. 19). This plasmid can be readily constructed from PCFM 836 (see U.S. Pat. No. 4,710,473 hereby incorporated by reference) by destroying the two endogenous NdeI restriction sites by end-filling with T4 polymerase enzyme followed by blunt end ligation and substituting the small DNA sequence between the unique ClaI and KpnI restriction sites with the small oligonucleotide shown below. The 5' to 3' sequence below is SEQ ID NO:96 and the 3' to 5' sequence below is SEQ ID NO:97.--

At Page 91-92, please replace the paragraph bridging pages 91-92 with the following paragraph, which has been amended to add sequence identifier information for the sequences following the paragraph:

--Plasmid V19.8 SCF$^{1-193}$ containing the rat SCF$^{1-193}$ gene cloned from PCR amplified cDNA (FIG. 14C) as described in Example 3 was digested with Bg1II and SstII and a 603 bp DNA fragment isolated. In order to provide a Met initiation codon and restore the codons for the first three amino acid residues (Gln, Glu, and Ile) of the rat SCF polypeptide, a synthetic oligonucleotide linker (sequences below 5' to 3' direction: SEQ ID NO:98 and 3' to 5' direction: SEQ ID NO:99)--

At Page 93, please replace the paragraph beginning at line 9 and ending at line 21 with the following paragraph, which has been amended to add sequence identifier information for the sequences following the paragraph:

--This example relates to the expression in E. coli of human SCF polypeptide by means of a DNA sequence encoding [Met^{-1}] human SCF^{1-164} and [Met^{-1}] human SCF^{1-183} (FIG. 15C); and [Met^{-1}] human SCF^{1-165} (FIG. 15C). Plasmid V19.8 human SCF^{1-162} containing the human SCF^{1-162} gene was used as template for PCR amplification of the human SCF gene. Oligonucleotide primers 227-29 and 237-19 were used to generate the PCR DNA which was then digested with PstI and SstII restriction endonucleases. In order to provide a Met initiation codon and restore the codons for the first four amino acid residues (Glu, Gly, Ile, Cys) of the human SCF polypeptide, a synthetic oligonucleotide linker (sequences below 5' to 3' direction: SEQ ID NO:100 and 3' to 5' direction: SEQ ID NO:101)--

At Page 145, please replace the paragraph beginning at line 19 and ending at line 26 with the following paragraph, which has been amended to add sequence identifier information for the sequences in the paragraph:

--pDSRα2 $hSCF^{1-248}$ was generated using plasmids 10-1a (as described in Example 16B) and pGEM3 $hSCF^{1-164}$ as follows: The HindIII insert from pGEM3 $hSCF^{1-164}$ was transferred to M13mp18. The nucleotides immediately upstream of the ATG initiation codon were changed by site directed mutagenesis from tttccttATG (SEQ ID NO:102) to gccgccgccATG (SEQ ID NO:103) using the antisense oligonucleotide

5'-TCT TCT TCA TGG CGG CGG CAA GCT T 3'(SEQ ID NO:104) --

At page 182, paragraph 1, please replace the paragraph between lines 5 and 28 with the following rewritten paragraph, which is presented to update the status of application referenced therein:

--Plasmid constructions for expression of numerous SCF analogs and fragments have been made. Site-directed mutagenesis had been used to prepare plasmids with initiating methionine codon followed by codons for amino acids 1 to 178, 173, 168, 166, 163, 162, 161, 160, 159 158 157 156 148 145 141 137, using the numbering of Figure 15C. The DNA for human SCF^{1-183} (Example 6B) was cloned into MP11 from Xba1 to BamH1.

Phage from this cloning was used to transfect an *E. coli* dut⁻ ung⁻ strain, R21032. Single stranded M13 DNA was prepared from this strain and site-directed mutagenesis was performed (reference IL-2 patent). After the site-directed mutagenesis reactions, the DNAs were transformed into an *E. coli* dut⁺ ung⁺ strain, JM101. Clones were screened and sequences as described in copending U.S. application Serial No. 717,334, filed March 29, 1985, now abandoned. Plasmid DNA preps were made from positive clones and the SCF regions from Xba1 to BamH1 were cloned into pCFM1656 as described in copending U.S. patent application Serial No. 501,904, filed March 29, 1990, now abandoned. The oligonucleotides for each cloning were designed to substitute a stop codon for an amino acid codon tat the appropriate position for each analog.--